

Effect of soil sieving on respiration induced by low-molecular-weight substrates**

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A b s t r a c t. The mesh size of sieves has a significant impact upon soil disturbance, affecting pore structure, fungal hyphae, proportion of fungi to bacteria, and organic matter fractions. The effects are dependent upon soil type and plant coverage. Sieving through a 2 mm mesh increases mineralization of exogenously supplied carbohydrates and phenolics compared to a 5 mm mesh and the effect is significant ($p < 0.05$), especially in organic horizons, due to increased microbial metabolism and alteration of other soil properties. Finer mesh size particularly increases arabinose, mannose, galactose, ferulic and phthalic acid metabolism, whereas maltose mineralization is less affected. Sieving through a 5 mm mesh size is suggested for all type of experiments where enhanced mineralization of low-molecular-weight organic compounds needs to be minimized.

K e y w o r d s: sieving, carbohydrates, phenolics, amino acids, microorganisms

INTRODUCTION

Sieving through 0.5-10 mm mesh sieves is used to remove fragments of litter, roots, fungal hyphae and stones, homogenizing soils in preparation for determination of microbial biomass C (C_{MIC}), microbial diversity, enzymatic activity, soil respiration, microbial uptake of labelled compounds and N mineralization (Devare *et al.*, 2007; Jan *et al.*, 2009; Nosalewicz and Nosalewicz, 2011; Jezierska-Tys *et al.*, 2011; Thomson *et al.*, 2010). Such preparation disturbs the natural soil pore structure with the loss of organic C when preferential flow cannot occur and heterogeneity of soil properties is reduced (ter Laak *et al.*, 2007). Soil disturbance due to sieving also results in accelerated C mineralization, N immobilization and denitrification (Sitaula *et al.*,

2000). Increasing soil disturbance through smaller sieves (0.5-2 mm) results in greater flush of easily utilizable C and N due to the disruption of aggregates protecting soil C, and creates new surfaces, which facilitates wetting of the disturbed material, leading to increased microbial growth and functional diversity (Marinari *et al.*, 2010). 2 mm sieves are often used to remove soil fauna (Blouin *et al.*, 2005), while 1 mm sieves are sometimes used to separate rhizosphere soil from the roots (Devare *et al.*, 2007).

Sieving inhibits mycorrhizal colonization, decreases the amount of fungal hyphae and disrupts the hyphae bound to soil particles and organic matter. The type of sieve has effects on the ratio of fungi to bacteria and increases the exposure of plant and microbial debris to decomposition processes (Thomson *et al.*, 2010). Disruption and removal of aggregates, which were stabilized by soil microorganisms, due to sieving depends on the type of plant coverage and phenolics content (Martens, 2000; Richardson *et al.*, 2012).

In this paper, we have attempted to compare the effect of sieving of fresh soil through commonly used 5 and 2 mm mesh sieves on mineralization of a range of low-molecular-weight (LMW) organic compounds which have different roles in microbial metabolism, including amino acids, carbohydrates and phenolics. Free amino acids in soil occur in concentrations of a μM to mM range, carbohydrates in soil solution and NaOH extracts in concentrations up to 358 mg kg^{-1} , and individual water-soluble phenolics in concentrations up to 600 mg kg^{-1} (Rejšek *et al.*, 2010; Vranova *et al.*, 2013).

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MATERIALS AND METHODS

Soils were collected in an old-aged spruce stand (103 years, spruce 90%, beech 10%, 480 m a.s.l., N 49°19', E 16°47', Haplic Cambisol) from the Oe horizon, in an old-aged beech stand (207 years, beech 99%, oak 1%, 480 m a.s.l., N 49°19', E 16°47', Dystric Luvisol) from the Oe and Aa horizons, in an old-aged mixed stand with a prevalence of deciduous trees (95 years, beech 52%, European ash 15%, larch 15%, spruce 9%, fir 5%, oak 2%, Douglas fir 1%, hornbeam 1%; 490 m a.s.l., N 49°19', E 16°40', Rendzic Humic Leptosol) from the Oe, Ahk, and Bwk horizons, in a middle-aged mixed stand of deciduous trees (33 years, oak 60%, hornbeam 30%, beech 10%; 415 m a.s.l., N 49°17', E 16°38', Haplic Cambisol) from the Ah horizon, and in arable land (220 m a.s.l., N 49°90', E 16°44') from the Ak horizon of Haplic Chernozem (IUSS Working Group WRB, 2006) (Table 1). Immediately after sampling, the soil was sieved through 2 and 5 mm mesh sieves and analyzed.

Mineralization of individual LMW organic compounds (amino acids, carbohydrates, and phenolics including salicylic acid) was performed by incubation of 10 g of mineral (or 5 g of organic) soil in 40 ml glass tubes with 2 mg of L- or D-amino acid (or phenolics) or 3.64 mg carbohydrate-C g⁻¹ dw to induce a maximum respiration rate (Rejsek *et al.*, 2010). Incubation was performed at 22°C to measure initial respi-

ration response for 6 h, the quantity of evolved CO₂ was determined using GC (YL6100, TCD detector, HP-Plot Q 30 m x 0.53 mm ID and 40 µm, 80°C).

Statistical analysis was performed by multi-factor ANOVA (Statistica 9.0) and the mean values were then compared by Fisher LSD test. A P value of ≤ 0.05 was used for indication of statistical significance.

RESULTS AND DISCUSSION

The effect of sieving through 2 versus 5 mm mesh was soil-dependent, showing, in most cases, non-significant (p>0.05) differences in mineralization of amino acids and significant (p<0.05) differences in mineralization of carbohydrates and phenolics (Tables 2, 3). In particular, the type of sieving had a significant (p<0.05) effect on mineralization of LMW organic compounds in Oe horizons of coniferous and deciduous forest stands, followed by A horizons of forests and arable land, with almost no effect on Bwk horizons. Mineralization of L-ornithine, L-valine, L-arginine, and L-aspartic acid was most affected from the range of tested amino acids and the same was found for arabinose, mannose, galactose, ferulic and pthalic acids from the range of carbohydrates and phenolics, respectively. Mineralization of some carbohydrates, such as maltose, was not significantly (p>0.05) affected by the type of sieving in all the tested organic and mineral soils.

Table 1. Selected physical and chemical properties of tested soils

Plot	C _{org} (%)	N _t (%)	C/N	pH _{H₂O}	pH _{0.01M CaCl₂}	Clay (%)	Silt (%)	Sand (%)
Spruce stand (old age, Haplic Cambisol, Oe horizon)	33.6	1.46	23.0	4.8	3.9	nt	nt	nt
Beech stand (old age, Dystric Luvisol, Oe horizon)	25.1	0.86	29.2	4.4	3.3	nt	nt	nt
Beech stand (old age, Dystric Luvisol, Aa horizon)	7.6	0.32	23.7	4.1	3.4	6.5	48.8	44.7
Deciduous forest (old age, Rendzic Humic Leptosol, Oe horizon)	26.2	2.00	13.1	7.7	7.2	nt	nt	nt
Deciduous forest (old age, Rendzic Humic Leptosol, Ahk horizon)	11.1	0.97	11.4	7.5	7.1	12.5	65.0	22.5
Deciduous forest (old age, Rendzic Humic Leptosol, Bwk horizon)	2.2	0.21	10.5	7.1	6.6	23.2	60.3	16.5
Deciduous forest (middle age, Haplic Cambisol, Ah horizon)	4.3	0.29	14.8	5.1	3.8	6.8	50.0	43.2
Arable land (Haplic Chernozem, Ak horizon)	4.3	0.17	25.9	7.7	6.9	19.7	48.6	31.7

nt – not tested.

Table 2. Amount of CO₂ (μ mol g⁻¹ dw) evolved from soil using different sieve mesh sizes and with addition of low-molecular-weight substrates over 6 h experiment (mean \pm SE, n = 3-9)

Compound*	Spruce stand (old age, Haplic Cambisol, Oe horizon)			Beech stand (old age, Dystric Luvisol)			Deciduous forest (old age, Rendzic Humic Leptosol)					
	Oe horizon			Aa horizon			Oe horizon			Ahk horizon		
	5 mm	2 mm	5 mm	5 mm	2 mm	5 mm	5 mm	2 mm	5 mm	2 mm	5 mm	2 mm
L-citrulline	1.80 \pm 0.20a	1.80 \pm 0.10a	0.70 \pm 0.03a	0.90 \pm 0.02b	nt	nt	0.70 \pm 0.02a	0.80 \pm 0.03a	0.70 \pm 0.02a	0.80 \pm 0.02a	0.70 \pm 0.02a	0.70 \pm 0.04a
L-cysteine	1.90 \pm 0.06a	1.90 \pm 0.05a	0.80 \pm 0.03a	0.90 \pm 0.02a	nt	nt	0.90 \pm 0.04a	0.80 \pm 0.08a	0.40 \pm 0.01a	0.40 \pm 0.01a	0.30 \pm 0.01b	0.30 \pm 0.01b
L-ornithine	1.00 \pm 0.10a	1.00 \pm 0.05a	0.80 \pm 0.05a	0.80 \pm 0.90a	nt	nt	0.60 \pm 0.10a	0.90 \pm 0.20b	0.20 \pm 0.001a	0.20 \pm 0.001a	0.10 \pm 0.004a	0.10 \pm 0.004a
L-valine	1.10 \pm 0.03a	1.30 \pm 0.10b	0.70 \pm 0.02a	0.70 \pm 0.02a	nt	nt	0.70 \pm 0.03a	0.90 \pm 0.01b	0.80 \pm 0.01a	0.80 \pm 0.01a	0.80 \pm 0.03a	0.80 \pm 0.03a
L-arginine	1.40 \pm 0.20a	1.80 \pm 0.03b	0.70 \pm 0.10a	0.90 \pm 0.20b	nt	nt	0.70 \pm 0.20a	0.80 \pm 0.10b	0.70 \pm 0.03a	0.70 \pm 0.03a	0.80 \pm 0.05a	0.80 \pm 0.05a
β -alanine	1.50 \pm 0.09a	1.20 \pm 0.30b	0.60 \pm 0.02a	0.70 \pm 0.02a	nt	nt	0.80 \pm 0.10a	0.80 \pm 0.10a	0.40 \pm 0.01a	0.40 \pm 0.01a	0.40 \pm 0.01a	0.40 \pm 0.01a
α -aminobutyric acid	1.50 \pm 0.04a	1.50 \pm 0.20a	0.70 \pm 0.02a	0.60 \pm 0.04a	nt	nt	0.50 \pm 0.03a	0.60 \pm 0.10a	0.40 \pm 0.01a	0.40 \pm 0.01a	0.40 \pm 0.02a	0.40 \pm 0.02a
L-alanine	1.80 \pm 0.10a	1.60 \pm 0.30b	0.70 \pm 0.03a	0.60 \pm 0.03a	nt	nt	1.00 \pm 0.03a	1.10 \pm 0.10a	0.50 \pm 0.03a	0.50 \pm 0.03a	0.60 \pm 0.03b	0.60 \pm 0.03b
L-aspartic acid	2.20 \pm 0.05a	2.60 \pm 0.05b	0.90 \pm 0.03a	0.60 \pm 0.03b	nt	nt	2.10 \pm 0.02a	2.10 \pm 0.10a	1.00 \pm 0.05a	1.00 \pm 0.05a	1.10 \pm 0.04b	1.10 \pm 0.04b
L-tyrosine	1.20 \pm 0.04a	1.20 \pm 0.08a	0.60 \pm 0.05a	0.70 \pm 0.001b	nt	nt	0.60 \pm 0.04a	0.70 \pm 0.01b	nt	nt	nt	nt
L-glutamine	2.00 \pm 0.02a	2.00 \pm 0.08a	2.20 \pm 0.04a	2.20 \pm 0.06a	nt	nt	1.30 \pm 0.10a	1.30 \pm 0.10a	nt	nt	nt	nt
L-glutamic acid	1.20 \pm 0.10a	1.20 \pm 0.10a	1.10 \pm 0.02a	0.90 \pm 0.02a	nt	nt	1.20 \pm 0.10a	1.20 \pm 0.10a	nt	nt	nt	nt
D-glutamic acid	0.8 \pm 0.20a	0.80 \pm 0.18a	0.80 \pm 0.05a	0.70 \pm 0.08a	nt	nt	0.60 \pm 0.09a	0.70 \pm 0.10a	nt	nt	nt	nt
D-alanine	0.9 \pm 0.05a	0.90 \pm 0.05a	0.50 \pm 0.30a	0.50 \pm 0.40a	nt	nt	1.00 \pm 0.10a	1.00 \pm 0.10a	nt	nt	nt	nt
Arabinose	0.5 \pm 0.03a	0.70 \pm 0.20b	0.70 \pm 0.04a	1.00 \pm 0.20a	0.40 \pm 0.08a	0.60 \pm 0.10b	2.40 \pm 0.10a	3.20 \pm 0.30b	0.40 \pm 0.07a	0.40 \pm 0.07a	0.50 \pm 0.09b	0.50 \pm 0.09b
Galactose	1.0 \pm 0.01a	1.30 \pm 0.20b	1.20 \pm 0.05a	1.90 \pm 0.20b	0.50 \pm 0.05a	0.70 \pm 0.09b	4.50 \pm 0.20a	4.30 \pm 0.40b	0.40 \pm 0.01a	0.40 \pm 0.01a	0.60 \pm 0.04b	0.60 \pm 0.04b
Mannitol	0.8 \pm 0.08a	1.00 \pm 0.03a	1.50 \pm 0.50a	1.50 \pm 0.50a	0.40 \pm 0.02a	0.60 \pm 0.08b	1.90 \pm 0.06a	2.50 \pm 0.08b	0.40 \pm 0.08a	0.40 \pm 0.08a	0.50 \pm 0.04b	0.50 \pm 0.04b
Mannose	0.6 \pm 0.05a	0.90 \pm 0.08b	0.60 \pm 0.04a	0.90 \pm 0.05a	0.30 \pm 0.03a	0.60 \pm 0.05b	1.50 \pm 0.09a	2.90 \pm 0.08b	0.20 \pm 0.02a	0.20 \pm 0.02a	0.50 \pm 0.08b	0.50 \pm 0.08b
Maltose	0.5 \pm 0.05a	0.60 \pm 0.04a	0.50 \pm 0.02a	0.80 \pm 0.03a	0.30 \pm 0.04a	0.40 \pm 0.04a	1.20 \pm 0.04a	1.40 \pm 0.05a	0.20 \pm 0.01a	0.20 \pm 0.01a	0.10 \pm 0.02a	0.10 \pm 0.02a
Sucrose	1.4 \pm 0.08a	1.50 \pm 0.10a	1.00 \pm 0.08a	1.20 \pm 0.10a	0.70 \pm 0.03a	1.50 \pm 0.09b	1.70 \pm 0.08a	3.00 \pm 0.10b	0.10 \pm 0.01a	0.10 \pm 0.01a	0.10 \pm 0.01a	0.10 \pm 0.01a
Fructose	1.0 \pm 0.08a	0.70 \pm 0.10a	0.60 \pm 0.02a	0.80 \pm 0.03a	0.40 \pm 0.001a	0.60 \pm 0.02b	1.10 \pm 0.10a	2.20 \pm 0.10b	0.10 \pm 0.01a	0.10 \pm 0.01a	0.10 \pm 0.01a	0.10 \pm 0.01a
Glucose	1.3 \pm 0.10a	2.70 \pm 0.10b	1.30 \pm 0.10a	2.20 \pm 0.08b	1.10 \pm 0.02a	1.10 \pm 0.01a	2.30 \pm 0.10a	2.90 \pm 0.10b	0.10 \pm 0.01a	0.10 \pm 0.01a	0.10 \pm 0.01a	0.10 \pm 0.01a

*2 mg g⁻¹ dw or 3.64 mg carbohydrate-C g⁻¹ dw. Explanations as in Table 1.

Table 2. Continuation

Compound*	Spruce stand (old age, Haplic Cambisol, Oe horizon)			Beech stand (old age, Dystric Luvisol)			Deciduous forest (old age, Rendzic Humic Leptosol)					
	Oe horizon			Aa horizon			Oe horizon			Ahk horizon		
	5 mm	2 mm	5 mm	5 mm	2 mm	2 mm	5 mm	2 mm	2 mm	5 mm	2 mm	
3-hydroxybenzoic acid	1.20±0.10a	1.90±0.10b	1.10±0.10a	0.60±0.02a	0.70±0.02b	0.80±0.02a	0.80±0.02a	0.90±0.03a	0.90±0.03a	0.80±0.02a	0.90±0.03a	nt
Benzoic acid	1.40±0.10a	2.40±0.20b	1.20±0.03a	0.60±0.01a	0.80±0.02b	0.90±0.06a	0.90±0.06a	1.30±0.04b	1.30±0.04b	0.90±0.06a	1.30±0.04b	nt
Ferulic acid	1.20±0.06a	1.60±0.09b	1.10±0.08a	0.50±0.01a	0.70±0.01b	0.70±0.03a	0.70±0.03a	1.20±0.03b	1.20±0.03b	0.70±0.03a	1.20±0.03b	nt
Pthalic acid	0.80±0.09a	1.70±0.05b	1.60±0.08a	0.60±0.02a	0.70±0.01b	1.30±0.01b	1.30±0.05a	1.70±0.05b	1.70±0.05b	1.30±0.05a	1.70±0.05b	nt
Adipic acid	0.80±0.02a	0.90±0.07a	1.70±0.04a	0.50±0.02a	0.50±0.03a	1.10±0.02b	1.30±0.01a	1.10±0.02b	1.10±0.02b	1.30±0.01a	1.10±0.02b	nt
4-hydroxybenzoic acid	1.80±0.02a	3.00±0.08b	0.90±0.01a	0.90±0.02a	1.40±0.1b	0.90±0.03a	0.70±0.03a	0.90±0.02b	0.90±0.02b	0.70±0.03a	0.90±0.02b	nt
Vanillic acid	1.00±0.02a	1.40±0.06b	1.70±0.05a	0.60±0.02a	1.00±0.10b	1.40±0.04a	1.40±0.04a	1.20±0.06b	1.20±0.06b	1.40±0.04a	1.20±0.06b	nt
Salicylic acid	2.70±0.10a	2.80±0.10a	3.90±0.20a	1.60±0.09a	1.30±0.05b	3.40±0.10a	3.40±0.10a	2.70±0.20b	2.70±0.20b	3.40±0.10a	2.70±0.20b	nt
Basal respiration rate	0.20±0.05a	0.30±0.05a	0.30±0.06a	0.10±0.02a	0.10±0.02a	0.30±0.06a	0.30±0.02a	0.30±0.02a	0.30±0.02a	0.30±0.02a	0.07±0.002a	0.07±0.002a

Respiration rates reported in this work are in accordance with other reports (Rejsek *et al.*, 2010). Sieving through a 2 mm mesh increased mineralization (Hassink, 1992). Soil sieving increases concentrations of NH_4^+ -N and NO_3^- -N due to release thereof from roots, and this change disrupts the natural microbial community structure and NO_3^- -N assimilation, including its subsequent use in biosynthesis (Rejsek *et al.*, 2010). Nevertheless, the type of sieving in the size range from <1 to 10 mm has generally a low effect on organic nitrogen forms and their stability (Rejsek *et al.*, 2010), suggesting small differences in mineralization of amino acids, as shown in this work.

Soil sieving increases stress on microbial communities, due to a flush of enzymatic activities and dissolved organic carbon, and reduces differences in substrate availability between soils (Gödde *et al.*, 1996; Hartley *et al.*, 2007; Turner and Romeo, 2010). Many researchers have failed to find any significant impact of sieving on C mineralization (Magid *et al.*, 1999; Persson *et al.*, 2000). Nevertheless, finer sieving through a 1-2 mm mesh increases C_{MIC} and soil respiration compared to soils sieved through a 4-5 mm mesh, indicating faster utilization of some carbohydrates in the finer mesh (Dorodnikov *et al.*, 2009), as found in this work.

Phenolics have little impact on soil pH, nevertheless, they react with positively charged hydroxides and, directly or indirectly, through inhibition of enzymes, they alter nutrient availability, including reduction of Fe or bound nitrogen into recalcitrant forms. Phenolics and salicylate are quickly metabolized, and salicylate supports metabolism of fungi, whereas phenolics supports bacterial metabolism. Decreased effects of phenolics on soil properties from A to B horizons was reported by Vranova *et al.* (2013).

CONCLUSIONS

1. Sieving through a 2 mm mesh sieves significantly ($p < 0.05$) increases mineralization of exogenously supplied carbohydrates and phenolics, especially in organic horizons, compared to 5 mm mesh sieving, probably due to increased microbial biomass carbon and support of metabolism of microbial groups.

2. This knowledge raises important issues that must be taken into account when preparing soils for experimental analysis, especially that which involves measurements of uptake of labelled low-molecular-weight compounds by plant roots when injected to soil and assessment of the effect of phenolics on plant growth and nitrogen sorption. In these cases, 5 mm mesh sieves will significantly minimize errors due to artificially enhanced mineralization, and should be used in preference to 2 mm mesh sieves.

Table 3. Amount of CO₂ (μ mol g⁻¹ dw) evolved from A and B horizons of deciduous forests and arable land using different sieve mesh sizes and with addition of low-molecular-weight substrates over 6 h experiment (mean±SE, n = 3-9)

Compound*	Deciduous forest				Arable land	
	old age, Rendzic Humic Leptosol Bwk horizon		middle age, Haplic Cambisol Ah horizon		Haplic Chernozem, Ak horizon	
	5 mm	2 mm	5 mm	2 mm	5 mm	2 mm
L-citrulline	0.30±0.02a	0.40±0.04a	0.20±0.01a	0.10±0.01b	0.10±0.02a	0.10±0.01a
L-cysteine	0.40±0.002a	0.40±0.02a	0.20±0.02a	0.20±0.02a	0.10±0.003a	0.10±0.004a
L-ornithine	0.30±0.001a	0.40±0.01b	0.20±0.04a	0.10±0.04b	0.10±0.01a	0.10±0.01a
L-valine	0.30±0.01a	0.30±0.01a	0.20±0.01a	0.10±0.01b	0.10±0.01a	0.10±0.01a
L-arginine	0.20±0.04a	0.20±0.01a	0.10±0.01a	0.10±0.005a	0.10±0.05a	0.10±0.05a
β-alanine	0.20±0.01a	0.20±0.02a	0.20±0.01a	0.10±0.01b	0.20±0.01a	0.20±0.01a
α-aminobutyric acid	0.30±0.02a	0.30±0.01a	0.10±0.004a	0.20±0.01b	0.20±0.01a	0.20±0.01a
L-alanine	0.40±0.01a	0.40±0.01a	0.20±0.004a	0.20±0.02a	0.20±0.01a	0.20±0.01a
L-aspartic acid	0.70±0.10a	0.70±0.10a	0.20±0.008a	0.20±0.007a	1.10±0.04a	1.10±0.04a
L-tyrosine	nt	nt	nt	nt	nt	nt
L-glutamine	nt	nt	nt	nt	nt	nt
L-glutamic acid	nt	nt	nt	nt	nt	nt
D-glutamic acid	nt	nt	nt	nt	nt	nt
D-alanine	nt	nt	nt	nt	nt	nt
Arabinose	0.50±0.07a	0.50±0.05a	0.40±0.09a	0.60±0.10b	0.30±0.06a	0.40±0.08b
Galactose	0.60±0.1a	0.50±0.07a	0.50±0.05a	0.70±0.04b	0.30±0.03a	0.50±0.01b
Mannitol	0.50±0.02a	0.60±0.01a	0.50±0.02a	0.60±0.03b	0.30±0.02a	0.40±0.03b
Mannose	0.30±0.04a	0.40±0.02a	0.30±0.01a	0.60±0.02b	0.30±0.05a	0.40±0.03b
Maltose	0.30±0.02a	0.40±0.02a	0.30±0.02a	0.40±0.04a	0.20±0.01a	0.30±0.03a
Sucrose	0.40±0.02a	0.40±0.02a	0.70±0.01a	1.40±0.02b	0.20±0.01a	0.50±0.02b
Fructose	0.30±0.01a	0.30±0.01a	0.40±0.01a	0.60±0.01b	0.20±0.02a	0.40±0.02b
Glucose	0.20±0.01a	0.20±0.01a	1.10±0.03a	1.10±0.03a	0.80±0.08a	0.80±0.07a
3-hydroxybenzoic acid	0.50±0.02a	0.70±0.01b	nt	nt	0.60±0.02a	0.60±0.02a
Benzoic acid	0.80±0.01a	0.70±0.01a	nt	nt	0.70±0.02a	0.70±0.02a
Ferulic acid	0.70±0.01a	0.90±0.01b	nt	nt	0.50±0.01a	0.60±0.01a
Pthalic acid	0.60±0.01a	0.70±0.01a	nt	nt	0.60±0.01a	0.40±0.01b
Adipic acid	0.80±0.01a	0.70±0.01a	nt	nt	0.40±0.01a	0.40±0.01a
4-hydroxybenzoic acid	0.60±0.01a	0.60±0.01a	nt	nt	0.30±0.01a	0.20±0.01a
Vanillic acid	0.90±0.02a	1.10±0.03b	nt	nt	0.60±0.02a	0.50±0.01a
Salicylic acid	1.00±0.02a	1.10±0.03b	nt	nt	0.40±0.02a	0.60±0.01a
Basal respiration rate	0.09±0.003a	0.09±0.003a	0.07±0.001a	0.07±0.002a	0.08±0.007a	0.08±0.006a

*2 mg g⁻¹ dw or 3.64 mg carbohydrate-C g⁻¹ dw. Different letters for the same soil indicate significant (p<0.05) difference between sieving through 2 versus 5 mm mesh. Explanations as in Table 1.

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